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COMMUNICATIONS

Physicochemical investigations of dipolar aprotic solvents as potential cholelitholytic agents

P. KENNELLY, O. I. CORRIGAN*, *Department of Pharmaceutics, School of Pharmacy, Trinity College, 18 Shrewsbury Road, Dublin 4, Republic of Ireland*

A number of dipolar aprotic solvents have been examined as potential co-solvents for gallstone dissolution. The power of these agents to solubilize such gallstone components as cholesterol, calcium carbonate, calcium palmitate and palmitic acid was determined and compared with that of monooctanoin (Capmul), using the synthetic solubility method. The solubilities of cholesterol and palmitic acid were greater in the *N*-methyl, *N*-ethyl and *N*-butylpyrrolidone derivatives than in monooctanoin. In contrast, the solubilities of the calcium salts were very low (<0·25% w/w) in all solvents examined. The influence of *N*-methylpyrrolidone (NMP) on both the in-vitro dissolution of cholesterol from gallstones and the decrease in stone weight with time was determined. NMP proved to be a better solvent than monooctanoin for human stones. NMP, which is miscible with water and monooctanoin, may have potential as a co-solvent in the design of solvent systems for gallstones.

Retained bile duct stones are present in 2–6% of patients who have had cholecystectomy and choledochotomy (Thistle et al 1980). The dissolution of such stones by instillation of solvents into the biliary system, via a T-tube, has been undertaken for many years (Pribam 1947; Admirand & Way 1972). The solvents used have either been aqueous bile salt solutions or non-aqueous solvents, such as monooctanoin (Capmul), the current solvent of choice, which has a high solubilizing capacity for cholesterol (Gadacz 1979; Flynn et al 1979). Wosiewitz et al (1983) reported that common bile duct stones have a lower cholesterol content than gallbladder stones, while therapeutic failure with monooctanoin has been linked to the presence of appreciable secondary stone components (Thistle 1983). The inhibiting effect of calcium bilirubinate, the principle pigment in stones, on cholesterol monohydrate dissolution in monooctanoin in-vitro has recently been confirmed (Mulligan & Corrigan 1986). In view of the disappointing results obtained with monooctanoin (Jarrett et al 1981; Allen et al 1985) a more rapidly effective solvent is desirable. Also cognizance must be taken of the solubility properties not just of cholesterol but also of the major secondary components.

Is this report we present the solubility behaviour of cholesterol, palmitic acid, calcium palmitate and calcium carbonate, all known important stone constituents (Sutor & Wooley 1971; Wosiewitz et al 1983), in monooctanoin and in a number of dipolar aprotic solvents. The latter solvents were chosen because of their unique solubilizing properties. They are generally good solvents for lipophilic materials and yet are miscible with aqueous media.

Materials and methods

Materials. Cholesterol monohydrate was prepared from anhydrous cholesterol (USP) in 95% ethanol as described by Flynn et al (1979). The product was stored under 100% humidity and protected from light. Differential scanning calorimetry (DSC) (Perkin-Elmer DSC-1B) and infrared (IR) spectroscopy were used to check the identity of the product. Calcium carbonate in the form of calcite was prepared as described by Reddy & Nancollas (1971). The identity of the product was checked by IR and X-ray diffraction methods. Palmitic acid (Sigma Chemicals) was used as received. Calcium palmitate was prepared by the method of Wosiewitz et al (1983). The solvents used were monooctanoin (Capmul 8210) supplied by Stokely Van Camp, Inc. Indiana, dimethyl sulphoxide (DMSO) supplied by Riedel-de-Haen AG Hannover, *N*-methylpyrrolidone (NMP), *N*-ethylpyrrolidone (NEP) and *N*-butylpyrrolidone (NBP) each supplied by GAF (Great Britain) Co. Ltd.

Solubility method. The 'synthetic' solubility method of Gordon & Scott (1952) also described more recently by Grant & Abougela (1982) was used to evaluate the solubility of pure gallstone constituents in the solvent systems.

Cholesterol assay. The cholesterol content of human gallstones was determined following extraction with

* Correspondence.

absolute ethanol using the CHOD-PAP enzymatic colorimetric method (Boehringer Mannheim GmbH Diagnostica). Cholesterol dissolution from stones into NMP vehicles was also quantified directly by the enzymatic method. Dissolution into monooctanoin vehicles was quantified by the above method as modified by Mulligan & Corrigan (1986).

In-vitro stone dissolution. In-vitro dissolution of human gallstones was investigated by the method of Gadacz (1979) also used by Thistle et al (1980). Eight pairs of matched gallstones (in terms of weight, composition, appearance, size and patient of origin) were chosen. These stones had an average cholesterol content of 81·4% (range 77·1–89·8). One stone from each pair was then placed in a 30 mL amber screw-capped jar containing 20 mL of monooctanoin and its match placed in a similar jar containing 20 mL *N*-methylpyrrolidone. The jars were closed and kept at 37 °C without agitation. At suitable time intervals up to 72 h the stones were removed from the medium, blotted dry, weighed and a sample of the medium taken for analysis.

Results and discussion

Solubility screening. The synthetic solubility method was initially used to estimate the solubility of cholesterol in monooctanoin at 37 °C. Plots of log solubility versus the reciprocal of the absolute temperature (i.e. Van't Hoff plots) were linear ($r > 0.995$) in each case. The estimated interpolated solubilities of anhydrous cholesterol in monooctanoin and monooctanoin containing 5% water were 12·95 and 15·81% w/w, respectively. The corresponding solubilities for cholesterol monohydrate were 14·21 and 14·77%, respectively. These results are consistent with the findings of Bogardus (1982) who observed cholesterol solubilities in the range 14 to 16% w/w in monooctanoin systems containing water up to 5% w/w.

Van't Hoff plots of cholesterol monohydrate solubility in NMP, NEP, NBP, and DMSO are compared with that in monooctanoin in Fig. 1. Cholesterol monohydrate solubilities at 37 °C were higher in the pyrrolidone derivatives and lower in DMSO than in monooctanoin. The solubilities at 37 °C in the pyrrolidone solvents increased as the alkyl chain length increased and were greater than those in other organic solvents quoted by Flynn et al (1979). The latter authors noted that as the alkyl chain length of the n-alkanols was increased cholesterol solubility likewise increased, though irregularly, up to a maximum at chain lengths of 6 to 7.

The estimated solubilities at 37 °C of the major gallstone constituents i.e. cholesterol, palmitic acid, calcium palmitate and calcium carbonate are summarized in Table 1. The dipolar aprotic solvents proved to be even more powerful solvents than monooctanoin for palmitic acid. Parker (1962) observed that dipolar aprotic solvents have affinity for large polarizable anions. In contrast to the lipophilic solutes, cholesterol

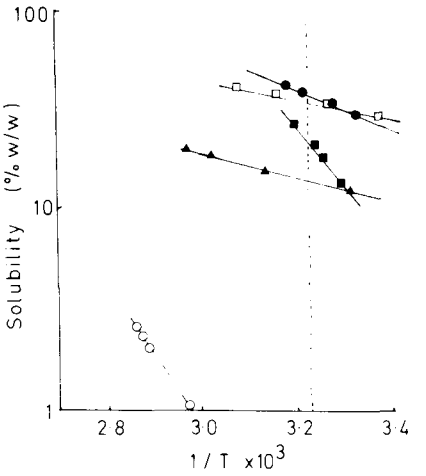

FIG. 1. Solubility-temperature relationships for cholesterol monohydrate in monooctanoin and dipolar aprotic solvents. Key: ▲ monooctanoin, ● *N*-butylpyrrolidone, □ *N*-ethylpyrrolidone, ■ *N*-methylpyrrolidone, ○ dimethyl sulphoxide.

Table 1. Solubilities (% w/v) of major gallstone constituents in monooctanoin and dipolar aprotic solvents at 37 °C estimated by the synthetic solubility method.

	Solute				
Solvent	Cholesterol (anhydrous)	Cholesterol (monohydrate)	Palmitic acid	Calcium palmitate	$CaCO_3$
Monooctanoin	12·95	14·21	*(2·36)	(0·23)	<0·03
Monooctanoin–H_2O (95:5)	15·81	14·77	(3·54)	(0·18)	<0·03
NMP	26·84	22·01	65·66	<0·03	<0·03
NEP	41·20	38·41	60·10	<0·03	<0·03
NBP	41·56	36·19	55·81	<0·03	<0·03
DMSO	(0·03)	(0·13)	81·44	<0·03	<0·03

* Values in brackets were obtained by extrapolation.

and palmitic acid, calcium palmitate and calcium carbonate have extremely low solubilities in the solvents examined. In most cases values were less than 0·03%, the lower limit of the solubility method employed.

Of the pyrrolidone derivatives examined, methylpyrrolidone is the least toxic and, in addition to having good solubilizing power for lipophilic stone constituents, it is miscible with aqueous media. Consequently methylpyrrolidone was selected for further investigation as a potential cholelitholytic agent or co-solvent.

Gallstone dissolution. The change in mean percentage weight loss with time of matched human gallstones in NMP is compared with that in monooctanoin in Fig. 2. After 3 h the mean weight of gallstones in monooctanoin had increased by 3·3%, indicating that the stones, all of which were still intact, had absorbed some of the solvent. In the same time the average stone weight loss

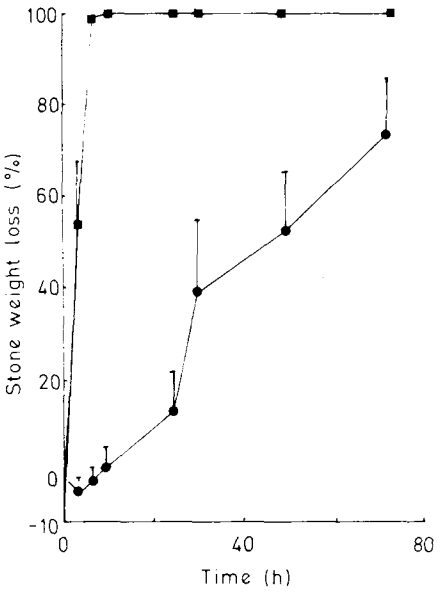

FIG. 2. Mean weight loss (%) with time of matched gallstones in *N*-methylpyrrolidone (■) and monooctanoin (●) at 37 °C. (n = 8, ± standard error).

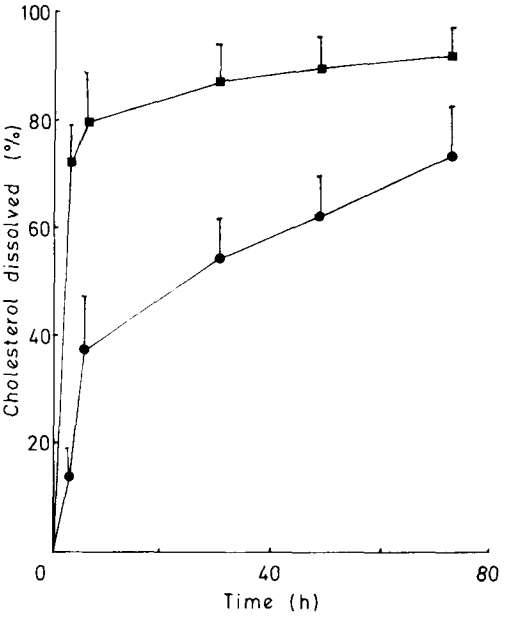

FIG. 3. Mean cholesterol dissolved (%) versus time from matched gallstones at 37 °C in *N*-methylpyrrolidone (■) and monooctanoin (●).

in NMP was 53·8%, though none of the stones had disintegrated. All stones for which a weight increase was observed in monooctanoin, contained less than 80% cholesterol. This initial increase in gallstone weight in monooctanoin was not observed by other workers (Gadacz 1979; Thistle et al 1980). However the initial weighing times in the current study were earlier.

After 6 h seven out of the eight stones in NMP had either dissolved completely or disintegrated into an unweighable sediment or sludge, giving an average percentage weight dissolved of 99·9%, while stones in monooctanoin still had an average weight increase of 1·7%.

At 9 h all stones in NMP had dissolved and/or disintegrated completely, while those in monooctanoin had achieved a weight loss of 2%. Mean weight loss in monooctanoin at 48 and 72 h were 52·4 and 74·9%, respectively.

Plots of percentage cholesterol dissolved versus time were also constructed (Fig. 3). These graphs show that cholesterol was released from both sets of stones after 3 h (13·2% from stones in monooctanoin compared with 71·6% from those in NMP). The initial stone weight increase and coincident release of cholesterol, in the case of stones in monooctanoin, indicates that while cholesterol was taken into solution in monooctanoin, the solvent was included in the stone structure to an extent that it could not be removed by blotting the gallstones. After 6 h, mean percentage cholesterol dissolved from stones in NMP was 79·1% while that of gallstones in monooctanoin was 37·0%. Cholesterol dissolution rate into NMP declined after 6 h, the percentages released after 30, 48 and 72 h being 87·0, 89·0 and 91·6%, respectively. Dissolution into monooctanoin continued slowly, giving values of 53·9, 60·8 and 72·4% at the corresponding times.

Plots of gallstone dissolution in monooctanoin obtained in this study (Figs 2, 3) were similar to those obtained by other workers (Gadacz 1979; Thistle et al 1980).

These results suggest that the dipolar aprotic solvent *N*-methylpyrrolidone, which is miscible with water and monooctanoin, may have potential as a co-solvent in the design of cholelitholytic solvent systems. Such water-miscible solvent systems would overcome the wetting problems associated with solvents which are immiscible with aqueous bile (Allen et al 1985) and would also permit the inclusion of water-soluble chelating agents known to dissolve the calcium salt constituents of stones (Nakamura et al 1978; Leuschner et al 1986).

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Studies on thyrotropin-releasing hormone-induced micturition in cats

DUŠAN B. BELESLIN*, DANICA JOVANOVIĆ-MIĆIĆ, RANKA SAMARDŽIĆ, BRANKA TERZIĆ, *Department of Pharmacology, Medical Faculty, P.O. Box 662, 11000 Belgrade, Yugoslavia*

In unanaesthetized cats micturition produced by thyrotropin-releasing hormone (TRH) was investigated after its injection into the cerebral ventricles through chronically implanted cannulae. TRH in doses from 0·1 to 1·0 mg evoked dose-dependent micturition. In cats treated with intracerebroventricular (i.c.v.) reserpine and 6-hydroxydopamine, but not with i.c.v. 5,6-dihydroxytryptamine and hemicholinium, the micturition caused by i.c.v. TRH was abolished. Chlorpromazine and antazoline injected into the cerebral ventricles prevented the micturition induced by i.c.v. TRH. On the other hand, mecamylamine, yohimbine, propranolol, atropine and methysergide injected i.c.v. had virtually no effect or partially antagonized the micturition evoked by TRH similarly injected. It is apparent therefore that centrally induced TRH micturition could be related to central catecholaminergic mechanisms.

Micturition has previously been described following injections of thyrotropin-releasing hormone (TRH) into the cerebral ventricles of cats (Metcalf 1974; Krstić et al 1983; Tomić-Beleslin et al 1985). However, little attention has been paid to the underlying neuropharmacological mechanisms. The present study was undertaken, therefore, to investigate the underlying neuropharmacological mechanisms of micturition produced by TRH injected into the cerebral ventricles of unanaesthetized cats.

Materials and methods

Cats of either sex, 2–4 kg, were anaesthetized using sodium pentobarbitone (35–40 mg kg^{-1}, intraperitoneally). Following aseptic precautions, a hole was drilled 7–8 mm from the stereotaxic zero line and 4–5 mm from the mid-line. A Collison cannula was then screwed into the calvarium, so the tip of the cannula rested in the left lateral ventricle (Feldberg & Sherwood 1953). The lower end of the cannula shaft was made of polyethylene tubing with a side opening 1 mm from its closed tip and positioned with the lumen facing the foramen of Monro. Postoperatively, penicillin was administered intramuscularly. An interval of five days elapsed after surgery before an experiment was begun. Post mortem dye studies indicated that the injected material passed from the lateral ventricle into the third and fourth ventricle.

Experiments for i.c.v. injections of TRH and pharmacological antagonists were carried out on cats of either sex, between 2–4 kg (n = 12). Successive injections of either TRH or pharmacological antagonists were separated by an interval of 72 h or longer. The specific regimen for the i.c.v. injections of TRH or pharmacological antagonists was randomized so that each animal was included in each of the experimental conditions. Each cat was used in 3–5 experiments.

In the separate series of experiments to study the effect of inhibitors of acetylcholine, catecholamines and 5-hydroxytryptamine synthesis on micturition evoked by i.c.v. TRH, cats of either sex, 2–4 kg (n = 24) were used. The effect of i.c.v. reserpine on the micturition response to i.c.v. TRH was evaluated over 24 h after a single injection of 1·0 mg, while the effect of i.c.v. 6-hydroxydopamine on the same response to i.c.v. TRH was evaluated over 10–14 days after two consecutive days of treatment with 6-hydroxydopamine in daily doses of 2·0 mg. Hemicholinium, 0·05 mg, was injected

* Correspondence.